REVIEW ARTICLE

Modern Approaches in Gene Therapy: Mechanisms, Vectors, and Therapeutic Perspectives

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Abstract

Gene therapy is the new approach to revolutionize the latest medical practice, to treat and prevent diseases by repairing or adjusting the malfunctioning genes on the molecular level. Gene therapy treats the cause of diseases, unlike conventional therapies, which mainly relieve the symptoms of the diseases, which presents an opportunity of long-lasting and therapeutic effects of the diseases. Gene delivery systems are quite effective and safe in regard to the efficacy of gene therapy. A wide range of viral vectors, including adenovirus, adeno-associated virus, lentivirus, retrovirus and herpes simplex virus, and non-viral vectors, including plasmid DNA, liposomes, polymeric nanoparticles and exosomes have been explored to increase the efficiency of gene transfer and reduce the toxicity and immunogenicity also, there are physical methods including electroporation, sonoporation, magnetofection, optoporation, gene gun and microinjection, which have enhanced localized gene delivery and therapeutic accuracy. Improvements in using gene therapy have been witnessed in cardiovascular diseases, cancer, and neurological disorders. Gene therapy stimulates angiogenesis and myocardial repair in cardiovascular diseases. Suicide gene therapy, microRNA-based therapy, nucleic acid therapy and oncolytic virus therapy are some of the approaches that have shown promise of tumor-targeting ability in cancer treatment. Gene therapy provides a possible cure in neurological diseases to counter the barrier of blood-brain and the low rate of neuronal regeneration. The sustained innovations in the development and targeting of vectors are widening the clinical potential of gene therapy and making it a promising therapeutic platform in the treatment of complex and hitherto untreatable diseases.

Key words: Cancer therapy, cardiovascular diseases, electroporation, gene delivery system, gene therapy, nanoparticles, neurological disorders, non-viral vectors, oncolytic viruses, viral vectors

INTRODUCTION

Gene therapy techniques used to transfer genes to treat or prevent disease are generally considered a future advancement in the medical field. A new direction in the genetic treatment of tumors is gene therapy, a scientific discipline that focuses on the regulation and repair of some genetic defects. Gene therapies also tend to affect the root causes of the diseases, unlike most of the existing medications, which largely revolve around symptomatic relief. The application of a gene-based method can potentially give superior targeting abilities and an extended length of action, thus causing a significant enhancement. This therapy, therefore, when compared to other forms of treatment,

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can cause subtle biological responses which are directly specific to the most relevant cellular organisms. This effect can eventually lead to significant improvements in the ratio of treatment and the rate of cure of diseases which currently do not have appropriate treatment options or are not properly treated. The ability of gene therapy to serve a wide variety of disorders with a single platform technology is an important strength of the technology. The implications of the collective results of gene delivery to the target tissue, implantation of new genetic material into cells, and the expression of the transfected gene within the target tissue determine the efficacy of gene therapy. When transfection is mediated, the use of specialized physical or biological targeting protocols frequently results in an increase in the gene expression in the target organ. The user has given a numerical sequence that includes the numbers 1 and 2. These developments in the field of gene therapy, both in its applications *in vivo* and *ex vivo*, as well as the growing clinical experience of the use of such tools, have been very instrumental in enabling the medical community to be able to achieve the full potential of the therapeutic capabilities of gene transfer. With the development of the sphere of gene therapy, it went beyond its original purpose of correcting hereditary diseases, which is why the field of application considerably broadened.[1] The use of various carriers in the gene therapy to treat various disorders is an attempt to reduce the side effects of the dosage forms being used, and at the same time improve the therapeutic efficacy in various diseases, were showed in Figure 1. Gene therapy using vectors has been prospective in the treatment of various diseases. Physical techniques help in the transfer of genetic messages through non-viral methods, or through the use of metal-produced nanoparticles to make targeted gene transfer.

GENE DELIVERY

In gene transfer, the effective transfer of genetic content to a particular site is a major element. Transfected gene is carried to the target organ in the presence of a certain biological or physical mechanism. Various techniques of delivering genes have been established in order to deliver a genetic intrusion in selected targets. All existing literature reports four mostly used gene delivery strategies allegedly common in the sphere of different gene treatments.[2]

1. Gene replacement
2. Gene modification is performed to make some changes to a given gene
3. Gene augmentation is the process of increasing or altering genetic contents to suitably enhance some of the characteristics or functions
4. The removal of the faulty gene.

GENE THERAPY IN CARDIOVASCULAR DISEASES

It is also demonstrated that gene therapy is used in the control of cardiovascular disease. The past few years have seen a tremendous spurt in the scholarly interest in cardiovascular gene therapy. The history of the development of therapeutic angiogenesis has been characterized by a slow and steady improvement. Gene therapy in cardiovascular medicine has great potential, and both inherited and acquired pathology have a special therapeutic intervention, which involves the use of gene therapy. Gene therapy is a new field that is promising in dealing with various cardiovascular diseases, including arteriosclerosis, heart failure, cardiac arrhythmias, angina pectoris, myocardial infarction and muscular claudication. This treatment method can enlarge the range of treatment methods that can be offered to patients with cardiovascular disease. Cardiovascular disease is often localized in nature, and gene therapy of the cardiovascular system is tailored to go to the specific localized areas only.[3]

GENE DELIVERY IN CARDIOVASCULAR DISEASE

Non-viral gene vectors

Plasmid DNA

Non-viral vectors are mostly considered as the safest option of therapeutic gene transfer. The use of plasmid DNA was very common during the early phase of the experimental and clinical research of the cardiovascular gene therapy due to the ease of its production and the high degree of safety. It has not been valid to use plasmid DNA in large trials of randomized controlled trials since gene transfer was poor.[4] The potential of these vectors is limited due to the naturally low expression levels with plasma DNA. The use of the matrix attachment region to improve the structural integrity of the plasmid DNA contributes to the improvement of tissue-specific enhancers, inhibition of untimely silencing of genes as a result of transgenic expression and the increase in the levels of transgenic expression facilitated by non-viral vectors. The current limitation on the efficacy of delivery of naked plasmid DNA to the vasculature can be improved by the use of a powerful secretory transgene, like vascular endothelial growth factor (VEGF). The first attempts on the use of synthetically produced liposomes to deliver naked DNA into the vasculature had poor efficacy and temporary gene expression. Different physical processes can boost the activity of plasmids by means of translocation of plasmids across cell membranes, for example, electroporation or ultrasonic treatment. According to the past research, percutaneous delivery of naked VEGF-2 plasmid DNA into the ischemic myocardium led to the shrinkage of the ischemic myocardium.

Antisense decoy oligonucleotides and small interfering RNA (siRNA)

The antisense decoy oligonucleotide has been used to suppress the pathways in the activation of cardiac and vascular diseases. Clinical research demonstrated that the efficacy of these antisense oligonucleotides was lower. This rise in

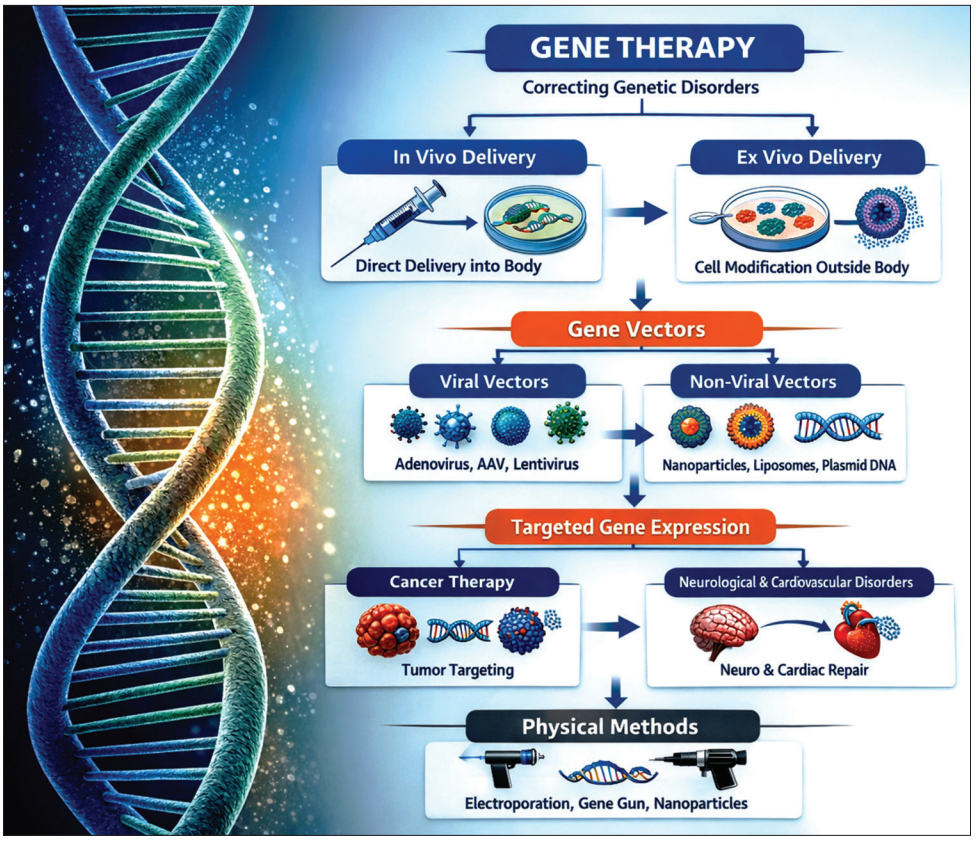

Figure 1: Gene therapy – revolutionizing genetic treatments

siRNA has resulted in either heightened or lasting silencing of the targeted specific genes. The above phenomenon has led to greater emphasis on the development of gene therapy of cardiovascular diseases.[5]

Viral gene vectors

Adenoviruses

It is clear why ad is the best method of gene transfer, especially *in vivo*, in a broad range of cell types. Ad vectors can apply genetic material to actively and quiescent cells in the body. Transgene expression has a strong degree of activity when delivered, but the level of activity is temporary due to the fact that the activity declines to low or no levels in most tissues within a period of 2 weeks. This phenomenon is explained by the fact that ad vectors have no capability of implanting themselves into the host genome, which hinders replication of the vectors as a precautionary measure against consideration of safety. The profile of the first-generation ad vectors is considered insufficient to correct diseases in the long run. These are the most common types of vectors that are typically used in clinical research of gene therapy. According to empirical evidence, the application of adenovirus vectors to the aim of expressing reporter and therapeutic transgenes within the cardiovascular system has proven to be effective in the research area of gene therapy. First-generation ads have a significant level of gene expression that has physiological consequences whose peak occurs a few days following gene transfer. These adenoviral vectors were immunogenic and hence caused the immune-mediated destruction of the vectors themselves and the transduced cells. The above limitation was overcome through the production of the gutless or helper-dependent adenovirus vectors. The application of vectors in cardiovascular gene therapy has been shown to have a lot of potential. Most clinical trials using adenovirus are based on vectors based on adenovirus serotype 5 (Ad5), an adenovirus species C that has been highly studied *in vitro* and *in vivo*. The Ad5 vectors can be easily produced to achieve the enhanced transfer of the gene in vascular cell that results in increased infectivity of adenovirus in the cultured vascular cells as well as intact veins in *ex vivo* experiments. This could be done by simple genetic modification on the fibre protein.[6]

Adeno-associated viruses (AAV)

Another very promising method is the use of AAV as a method of vectors. AAVs are a form of single DNA viruses which require helper viral activity to replicate. AAV has a relatively simple genome containing two open reading frames, one of which encodes non-structural replicative proteins, and the other one encodes structural capsid proteins. These open reading frames are bound by two hairpin structures known as internal terminal repeats. AAV can be used to insert itself into the genome of the target cell. Adeno-associated bacteria vectors have shown lesser immunogenicity to regular viral vectors used as vectors in gene therapy. Moreover, these

vectors have innate capacity to maintain expression of genes after successful transfer of the gene to the target cell. The adeno-associated viral vectors were shown to be very promising in gene transfer and permanent gene expression within the cardiovascular system. AAV vectors have been well-documented to be effective in transducing the blood artery walls, skeletal muscle, the heart, and the liver, and as such, they are highly suitable in cardiovascular gene therapy of these organs. The efficacy of bare plasmid DNA in the myocardium is significantly lower than that of their counterparts, which are several orders of magnitude more effective. AAV possesses a number of features that can be applied to the sphere of gene therapy with certain benefits. The specified phenomenon displays an intrinsic tendency towards the vascular smooth muscle cells, the cardiac myocytes, and skeletal muscle. It successfully transforms dormant cells into active ones and triggers sustained gene expression, which lasts over a long period and usually a considerable duration of many months. A previous study has reported that the correction of the metabolic phenotype was partially corrected by the transfer of the very low-density lipoprotein receptor gene using AAV2. Little was done to improve the efficacy of gene transfer until the development of pseudo-type AAV vectors, which entailed the encapsulation of the AAV2 genome in the capsids of other serotypes such as AAV7 or AAV8. The delivery of *LDLR* gene into LDLR-deficient mice with the help of the new AAV7 and AAV8 serotypes resulted in a significant and long-lasting decrease in the level of cholesterol in the bloodstream. Furthermore, these animals never showed the increased progression of severe atherosclerosis that was seen in untreated mice. The tolerability of AAV-based on human pathology is a significant benefit of AAV-based vector systems. Latent AAV infections are widespread among the human population, but there is no such disease reported in the victims.[7]

MYOCARDIAL GENE DELIVERY SYSTEMS

Needle injections

The application of the direct injection needle of the vector is a simple and easy method of conveying genes to the myocardium. The existing method is inefficient since it often leads to the identification of transduced cells only in the area of the needle path. Moreover, transgenes are often inhibited due to the low concentrations, which are primarily explained by the high rate at which the vectors are cleared. This is further worsened by an inflammatory response that is triggered locally due to tissue injury occasioned by the insertion of the needle. Microneedles (MNs) are a series of tiny needles, with a maximum length of 1 mm, that offer a safe method of the delivery of therapeutic agents, especially macromolecules, with minimal incidences of skin injury or pain. The macromolecules include the nucleic acids, which come in the form of genes, vaccines and proteins. MNs delivery of therapeutic pharmaceuticals presents a promising and efficient method of delivering treatment to ischemic heart disease (IHD). MNs allow proper and effective delivery of the drugs, their even distribution throughout the body. This special form of administration has great prospects in the management of IHD.

Catheters

Numerous intracardial catheter systems that enable the injection of 10–100-l volumes of vector formulation have been used for myocardial gene transfer in large animal models. These devices frequently exhibit superior vector retention at the delivery site in comparison to transepicardial needle injections. Catheters come in a variety of forms, such as channel balloon catheters, hydrogel-coated catheters, and microporous catheters. The gene can be delivered to the vessel wall by these. Blood vessels are already perfused or altered by a number of common operations. Intravascular gene transfer is therefore a promising treatment option for heart conditions. To solve this problem, nipple catheters or needle injections from the inside of the vessel have been created for direct gene delivery into the arterial wall.[8] The first to be utilized were double-balloon catheters, which allow fluids carrying vectors to be injected by creating a space between them. Even though this approach has demonstrated consistent, efficient adenoviral vector delivery to the arterial wall, complete artery closure for prolonged periods of time may result in problems du to downstream ischemia. Infiltrator catheters allow vector injection into the vessel wall by tiny injection a needle, which reduces the likelihood of vector systemic dissemination and may increase transgenic medial delivery.

Vascular delivery systems

For the objective of cardiac gene transfer, different intracardial catheter methods have been used in large animal models to enable the injection of 10–100 L of vector formulation. These devices often show better vector retention at the site of delivery compared to transepicardial needle injections.[9] There are currently several different kinds of catheters, including channel balloon, hydrogel-coated, and microporous catheters. These substances have the capacity to carry the gene to the blood vessel's inner lining. Numerous standard surgical techniques already entail the perfusion or modification of blood vessels. Consequently, a viable therapeutic strategy for the management of cardiovascular disorders is intravascular gene transfer. To address this issue, innovative approaches such as needle injection from the inside of the channel and nipple catheters have been developed to allow direct gene delivery into the artery wall. The early use of double-balloon catheters involved creating a gap between the two balloons to permit the infusion of fluids carrying vectors. While this approach has demonstrated the continuous and successful delivery of adenoviral vectors to the artery wall, prolonged arterial blockage is likely to cause difficulties due to downstream ischemia. Infiltrator catheters,

which use small injection needles, make vector injection into the vessel wall easier. This method lowers the chances of vector systemic dispersion and could improve transgenic medial administration.

Gene therapy in cancer therapy

Cancer is universally recognised as a serious and life-altering health illness. Cancer can be produced by a variety of variables, including physical, chemical, microbiological, immunological, and genetic. Potential causes include genetic anomalies in any gene that encodes cell cycle proteins, as well as somatic mutations in upstream cell signaling pathways. Despite the increased availability of standard cancer treatment options, a large number of people die from cancer each year. Different cancer therapy strategies have been developed dependent on the stage of the tumor. Cancer diagnosis and staging play an important role in supporting successful disease management and treatment. A large body of clinical research has proved gene therapy's efficacy as a potential cancer treatment. Numerous studies have been undertaken to explore gene therapy as a viable cancer treatment alternative. Several techniques, including bare plasmid or DNA injection, oncolytic *in vivo* therapy focused on microRNAs, tumor suppressor gene integration, and gene-guided enzyme pro-drug therapy, are used to selectively target diverse types of malignant tumors. The efficacy of cancer gene therapy is dependent on the use of a vector that is safe, effective, and regulated. The initial proposal for a gene therapy platform was to use viral vectors. To overcome this challenge, nanomedicine has developed a plethora of new platforms. The bulk of these systems makes use of nanoscale structures, specifically nanoparticles. The suicide gene strategy, together with oncogenes and tumour suppressor genes, can be used to combine chemotherapy and gene therapy. The suicide gene causes the development of an enzyme that is not found in mammals. This enzyme is utilized to transform a non-poisonous prodrug into a cytotoxic metabolite that acts particularly on malignant cells. The method used in this work is based on the transformation of a biocompatible precursor molecule into a strong cytotoxic derivative exclusively within malignant cells, were showed in Figure 2. The conversion process involves the overexpression of a non-mammalian enzyme in neoplastic cells as a result of effective gene transfection.[10]

GENE DELIVERY IN CANCER TREATMENT

Nucleic acids-based gene therapy

Application of naked plasmid DNA to treat cancer has gained much attention as it has superiority in terms of safety profile and exerting less effort as compared to the traditional approach. The length of the naked DNA vectors plays a big role in their survival in human serum with longer and heavier vectors having short periods of survival. Consequently, many of the scientists came up with minute vectors in an attempt to enhance their life span in human serum. The inhalation of the naked DNA particles caused a significant increase in the expression of the genes in the pulmonary system, as well as decreased the proliferation of the lung cancer cell types. There is a possibility of aerosol delivery of naked and unmodified new RNA interference (RNAi) medications as potentially fascinating in the treatment of lung cancer, particularly when there are no delivery vehicles. High-pressure gene cannons or inhalation can be used to deliver bare DNA to the targeted

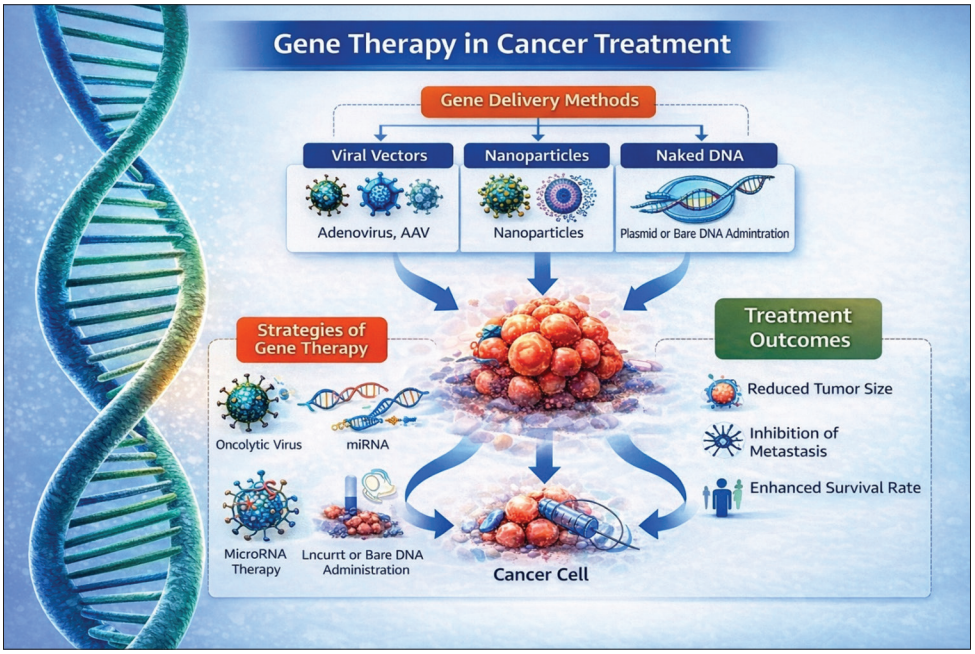

Figure 2: Gene therapy in cancer treatment

cells, including skin tumors or cancerous cells in the lungs. Elsewhere, researchers have developed DNA liposomes that comprise pigment epithelial-derived factor genes, and these have been demonstrated to be effective in preventing tumor growth and metastasis. The genes were placed in liposomes as gene therapy, especially that of colorectal cancer. The researchers determined that cancer-targeted liposomes were significantly more effective in inhibiting multiple cell events, such as invasion, migration, and induction of apoptosis, when it was analyzed *in vitro* on colorectal cancer cells. The expression of the gene-loaded liposomes led to an increase in the survival duration of the laboratory mice with metastatic colorectal cancer, as well as a reduction in the tumor nodules in the lungs. The murine *interleukin (IL)-12* gene, without any protective covering, was transferred into mice and demonstrated strong gene expression despite a single injection and had a huge anti-cancer effect comparable to many days of repeated intraperitoneal therapy of recombinant murine IL-12. A significant benefit of bearing transfer of *DNA* gene in skeletal muscle is that it is capable of inducing long-term expression. Electroporation is a technique, which enhances the uptake of uncomplexed DNA after intramuscular injection.[11]

Suicide genes

This method relies on the technology involving the use of non-mammalian-derived enzymes that facilitate the transformation of non-hazardous chemical molecules into biologically active compounds having physiological outcomes. The overexpression of the enzymes in the neoplastic cells occurred after successful gene transfection. The suicide gene system, comprising of herpes simplex virus (HSV) thymidine kinase and ganciclovir, has received a significant amount of scholarly attention. One of the strongest characteristics of these systems is the bystander effect. This is a process in which transduced cells pass on harmful chemicals to the surrounding cancer cells through gap junctions and apoptotic vesicles.[12] There are various types of cancer that suicide gene therapy has been researched on, such as colon, liver, lung, medulloblastoma, neuroendocrine and spinal cord tumors. Furthermore, the research has been done on prostate, breast, bladder, brain, gliomas, head and neck, sarcomas, and ovarian cancers. Suicide gene therapy, one of them, is a comparatively novel approach in this field. The technology that was used consisted of the use of a genetically engineered version of caspase-9, which was used to initiate the process of programmed cell death, also referred to as apoptosis. The chemical compound AP1903 was used to conjugate the inactive form of inducible caspase-9 (iCasp9) and FK506-binding protein FKBP12 in the study by the researchers. This interaction allowed apoptosis by dimerization only. Dimerization was observed after AP1903 injection, and this led to the fast death of cells.[13] On-site administration at the tumor site has been investigated and is being used as an attempt to enhance suicide gene therapy. This study revealed no notable histological negative effects on lung cancer patients or cancer cell lines of glioma. Nevertheless, the basis of the municipal government is not always feasible. Consequently, there has been much research on the Trojan horse strategy. It is founded upon the endocrine evasiveness of tumor-tropic neural stem cell (NSCs) (also known as NSCs) to roll out gene expression products through non-viral vehicles continuously. New compounds are being designed which are less toxic and have the capacity to carry a high DNA loading capacity. Many innovative approaches to directed suicide gene therapy are underway, along with the creation of innovative promoters of overexpressed genes. The application of suicide gene therapy has been proven to be effective in cases whereby the therapeutic formulation is administered afar of the initial disease effectively controlling the primary lesion. This observation is true with regard to the management of remote metastases. The gene therapy on the suicide genes has been found to enhance the immune activity not only in the immediate vicinity, but in remote sites of metastasis. The synergistic effect of these two expressing of the double-suicide gene made the cytotoxicity more effective through the enhancing cell necrosis. The unpredictable induction of apoptosis in advanced or metastatic colon cancer is treated in this way. The study of the DLD-1 colon cell line indicated that a combination of the presence of the suicide genes and apoptin led to a dramatic reduction in the growth of the cell.

TARGETING MICRO RNAS BASED GENE THERAPY

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ONCOLYTIC VIRUS (OV) THERAPY

OVs have the peculiarity to infect neoplastic cells selectively, without promoting injury to the normal cells. The proliferation of the virus in the tumor makes the tumor eradication process easier. Talimogene laherparepvec, an OV constructed from herpesvirus, has been genetically engineered to express granulocyte-macrophage colony-stimulating factor and is approved to be used in 2015 by the United States Food and Drug Administration. It was an important achievement since it was the first OV to be formally approved as a cancer therapeutic agent to treat melanoma in the United States. As a result of the first clinical trials (7–9) done on OVs, there was evidence of promising safety profiles even in high doses. OVs have the beneficial factor of the tendency to have a strong replication and proliferation within the cytoplasm of cancerous cells, but do not integrate with the human genome. This property improves their safety profile, their therapeutic efficacy and makes them more appealing as agents of cancer treatment. It presented a novel formulation in their study that entailed the use of targeted nanoparticle to deliver a neutral vesicular stomatitis virus matrix protein gene, and the study was intended to examine its potential in the treatment of cancer. The researchers claim that the formulation had a high degree of compatibility with blood and demonstrated the possibility of specific targeting of tumors. The formulated formula proved to effectively target the cancerous cells; as a result, melanoma growth decreased, and the spread of tumors was prevented, after intravenous administration. This intervention, therefore, led to a substantial increase in the time span of mice with the tumor to survive. According to the results of the above-mentioned scientists, the anticancer effects of the formulation consisted of stimulated apoptosis, inhibited angiogenesis, and the provocation of virus-related signal pathways.[16] Adeno-associated vector has been considered to be the best viral vector to use as a cancer therapeutic agent due to its non-pathogenicity nature, the ability to transduce both dividing and non-dividing cells and the lower immunogenicity compared to other viral vectors.

GENE THERAPY IN NEUROLOGICAL DISORDERS

The neurological system is hard to research, and the brain is the genesis of many of the most widespread illnesses. The metabolism, brain development, and functioning may be impacted by a wide variety of disease illnesses, both globally and locally. The treatment of these diseases has hardly been assisted by medicines and neurosurgery due to the lack of pathophysiology information and complexity of it. The blood-brain barrier (BBB) also restricts systemic therapy in that therapeutic chemicals cannot reach the central nervous system (CNS). The neurological conditions of the CNS include Alzheimer's, Parkinson's, Huntington's, amyotrophic lateral sclerosis, and stroke, which are challenging to cure using the standard pharmacological approach due to late diagnosis. The symptoms and effects of the diseases will be lifelong to the patients. One of the alternatives is promising, and it is the gene therapy that introduces DNA into the CNS. As preclinical information increased, there has been gene therapy research done in various diseases of the nervous system. The CRISPR technology has created an interest in the application of genome editing to cure various diseases. All these are perfect in monogenic diseases that can be treated by remediating the mutation. These illnesses encompass neurodegeneration, aberrant cellular activity, cancer, neuroinflammatory, metabolic and developmental problems that may impact on development, metabolism and functioning on the global or local levels. Neuro disease, lack of knowledge and its complexity are the main cause of failure in medication therapy and neurosurgery surgeries. Since the ageing of the population has the tendency to increase over time the disorders of the nervous system, the existing medicines and other possible alternatives should be enhanced.[16,17] More research expounds on the use of gene therapy in the management of neurological disorders, including substituting the defective gene with a functional counterpart or silencing the gene. RNAi is the most popular method of silencing the production of target genes. The short non-coding RNA of different lengths is bound to a similar region of the mRNA

target. Therefore, gene therapy in neurological therapy assists in overcoming neurological crises and developing extensive treatment options, were showed in Figure 3.

VECTORS USED IN THE TREATMENT OF NEUROLOGICAL DISORDERS

Viral vectors

Gene therapy is premised on safe and effective vectors to transfect human cells using therapeutic genetic content. Advances in gene therapy have resulted in improved designs of vectors and safety profiles of the targeted genes delivery and concrete effects. Recent methodologic advances comprise accurate viral vectors design, plasmid transfection, nanoparticles, polymer-mediated gene delivery, customized microRNA and *in vivo* CRISPR-based therapy. The key benefits of viral vectors are their high level of cell type specificity and stable transmission. The transgenic long-term expression can be provided through retroviruses and lentiviruses when they are integrated. They have the risk of activating oncogenes, and the alteration of normal genes, such as tumor suppressors. The ideal vectors in use are those that are episomal and are used in peripheral nerves.[18-20] The lentiviruses, as well as retroviruses, have the ability of transferring novel genetic material into the host cell chromosome, avoiding losing the therapeutic genes in the growing cells. Retrogradely delivered, adenoviral vectors do not require integration, but expression of the viral genes triggers an inflammatory response, and they do not linger in sensory neurons. AAVs have the ability to deliver genes to the brain. They are not harmful as they can reproduce with helper viruses such as the HSV and adenovirus. Compared to the traditional types of AAV serotypes, different viral serotypes tend to infect different cell types. The new AAV serotypes transduce neurons differently, but AAV1, 5, and 8 might be effective transducers of glial cells. Nevertheless, there are a number of neuronal-specific promoters whose transgenic expression is only restricted to neurons. AAV has the capability of transducing nerve tissue, retrogradely transferring transgenes (in mice) and long-term expression of transgenes. The size of their payload (3–4 kb) is small enough to only transport a single moderate-sized gene at an adequate dosage, and the dosage of viruses needed to achieve transduction is more abundant compared to other vectors.[19] Lentivirus is a retrovirus that is encapsulated in the form of a single-stranded RNA and generates a number of effector molecules. It becomes part of the host genome and years of therapeutic genetic expression. Its carrying capacity is 8–9 kB. Transfection of dividing cells can be done using integration; however, it may induce insertional mutagenesis in regions of the genome not intended. HSV is an enclosed double-stranded DNA virus, which can host to 40 kB of genetic material, but with the lowest tissue penetrance and bio dispersion. Its key strengths are the capability of HSV to become dormant as a nuclear episome following infection and its affinity toward transfecting the CNS neurons, especially the sensory neurons.[21]

Non-viral vectors

The transfection vectors used in gene therapy should possess the following characteristics: they are required to protect the nucleic acid against blood enzymes, endonucleases, stimulate internalization of the nucleic material into target cells and release the nucleic acid at the target site of the cell. Polyethylenimine (PEI) forms homogenous DNA nanoparticles, thus it is the most effective

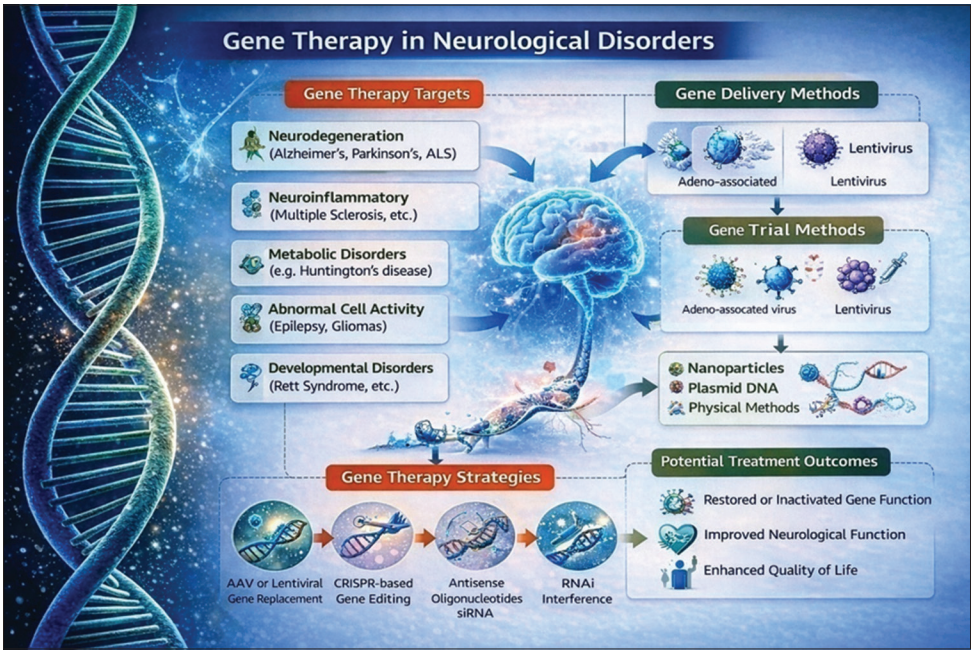

Figure 3: Gene delivery in neurological disorders

cationic polymer in most cell types. PEI forms uniform polyplexes with negatively charged DNA due to its high positive charge density. The DNA is then conjugated with PEI to create a nanoparticle, which is spherical and can be endocytosed by the cell. Add positive charge to the polyplex, incubation time and PEI-DNA concentration to enhance endocytosis. Once the cell is invaded the polyplex becomes endo-lysosomal. Another good cationic polymer in gene transfer is poly (L-Lysine) because it is both biodegradable as well as non-cytotoxic. Brain gene therapy is made by developing biodegradable polymers. These are disulfide-bonded biodegradable polymers. The polymers remain intact outside of the cell and are rapid cleaved within the cytoplasm due to the redox potential difference between the oxidizing environment outside of the cell and the reducing environment inside the cell. Therefore, bio-reducibility can decrease cytotoxicity and subcellular release of genetic drugs. The 3D topological structure of Branching PBAE provides more chemical surface area to be functionalized, so it is superior to genetic drug loading and protection. It has been used to transfect multipotent adipose-derived stem cells and astrocytes that have low levels of transgenes expression. Inorganic NPs can be used to deliver genetic drugs through iron oxide, gold, carbon nanotubes, and silica to the brain diseases. The greatest of their advantages is the facile size control and easy surface modification of inorganic NPs. Inorganic nanoparticles that have fluorescence, iron oxide that has magnetic resonance, and gold that has X-ray computed tomography are advantageous in order to monitor bio-distribution and direct therapy. Genetic medication delivery is commonly done using gold nanoparticles because they can be controlled in size and are easy to produce. Exosomes are endocytic extracellular vesicles of 40,120 mm. Different types of cells release them into blood, urine and cerebrospinal fluid. Exosomes are membrane lipid vesicles which have layers of phospholipid and surface proteins. Proteins and nucleic acids can be found as bioactive molecules within it. They are used as non-viral vectors, and they are carrying genetic drugs. The exosomes are superior to synthetic NPs due to their origin and constituents. Exosomes are characterized by low toxicity and biodegradability. Exosomes can evade opsonins, coagulation factors and complement due to the expression of CD55 and CD59. Exosomes are not recognized by the immune system, thereby they remain long in circulation. Exosomes have the ability to penetrate tissue and cellular barriers in the case of brain gene therapy.[22]

Receptor-mediated gene delivery

Although the mechanism between the cell surface and the nucleus is not known, transport of genes by receptors can occur through endocytosis, endosomal escape, and entry into the nucleus, followed by transcription. Exosomes are endocytosed and transcytosed by cell type-specific protein receptors of BBB endothelial cells. Particular cell-surface receptor complexes interact with antibodies or transferrin in order to get into cells through receptor-mediated endocytosis. Gene could be directed to the specific receptors by antibodies or fragments of antibodies. Considering the success of antibodies as a treatment recently, it is surprising that so little research has been done in their specific targeting to drop genes into neurons.[23,24] Investigators were able to readily deliver genes to the neurons in the spinal cord and the brain with specific immune genes generated out of antibodies of significant receptors.

Physical techniques for the gene delivery

The physical techniques will aid in the transfer of the genetic material into the desired tissue minimizing breakdown of the material and carriage. There is increased efficacy of treatment and on-site rapid activity. This enhances the transfer and delivery of genes.

MECHANICAL METHODS

Microinjection method

The simplest method of delivering DNA into cells is microinjection into the nucleus or cytoplasm. This microsurgical technique involves the use of glass needle, accuracy positioning device and micro injector to act on one cell. Bare DNA microinjected into the nucleus was not broken in the cytoplasm, and gene expression was maximized compared to intracytoplasmic injection.[25] Nucleic acids can be transferred directly to any type of cell using MNs without any barriers to gene delivery, but only single-cell transfections are ineffective when using MNs in most tissue engineering. Microinjection is a very delicate procedure that needs accuracy and accuracy, and thus, skill becomes a determinant to its effectiveness. The shape, size and position of target cells also have a possibility of interfering with microinjection transfection. Training needed: Cell separation and immobilization: this needs further training to transfect cells using microinjection. Tissue engineering by microinjection could be more attractive in case the process of cell isolation and microinjection can be automated, and the human factor is removed. The easiest way of delivering genes is through microinjection, but it is hard to carry out. The highly successful pronuclear DNA injection method is time-consuming since only a single cell can be injected at a time; thus, transfection is only achievable on a small number of hundreds of cells at a time. Therefore, microinjection is not feasible in the majority of *in vivo* applications of gene transfer at current technology.[26]

Gene gun method

Particle bombardment gene-mediated a carrier of accelerated particles can be used to deliver bare DNA plasmids to target cells. These small particles have the ability of precipitating naked DNA and releasing it into the cell after bombardment.

The former employed gunpowder acceleration through the use of DNA-coated tungsten particles that were used to carry DNA across the plasma membrane and deliver genes. Since, numerous gadgets are produced since then, the most popular ones are gold beads. The most popular in the market are Accell and Helios gene guns. This delivery method is dependent on DNA loading and particle size, as well as the delivery time. The DNA-coated beads are dispersed based on the acceleration control of the gene cannon. In ballistic gene delivery, the gene of interest may be transfected into non-targeted cells within the gene cannon dispersion region since the method lacks cell selectivity. Moreover, small particles are only able to carry a small amount of DNA or RNA. Tissue engineering by ballistic gene delivery involves several treatments to transfect a large number of cells. It has not been established that there is an effective way of making sure that large numbers of treatments spread DNA micro particles uniformly without causing inflammation of the target tissue.[27]

Physical methods

Electroporation

Particle bombardment gene-mediated a carrier of accelerated particles can be used to deliver bare DNA plasmids to target cells. These small particles have the ability of precipitating naked DNA and releasing it into the cell after bombardment. The former employed gunpowder acceleration through the use of DNA-coated tungsten particles that were used to carry DNA across the plasma membrane and deliver genes. Since numerous gadgets are produced since then, the most popular ones are gold beads. The most popular in the market are Accell and Helios gene guns. This delivery method is dependent on DNA loading and particle size, as well as the delivery time. The DNA-coated beads are dispersed based on the acceleration control of the gene cannon. In ballistic gene delivery, the gene of interest may be transfected into non-targeted cells within the gene cannon dispersion region since the method lacks cell selectivity. Moreover, small particles are only able to carry a small amount of DNA or RNA. Tissue engineering by ballistic gene delivery involves several treatments to transfect a large number of cells. It has not been established that there is an effective way of making sure that large numbers of treatments spread DNA micro particles uniformly without causing inflammation of the target tissue.

Sonoporation

Sonoporation is heightened by ultrasound. Sonoporation is used to treat cardiovascular tissue, breast cancer, liver cancer, pancreatic cancer, endothelial cells and renal tubules. There are a number of frequencies and waveforms of ultrasound, but most studies have been carried out on sonoporation using sinusoidal probes at megahertz.[28] High-intensity focused ultrasound induces localized shear stresses within the extracellular fluids and stimulates all of the cell membrane growth and membrane permeability to plasmid DNA and drugs. The ultrasonic contrast agents, such as Optison™ have the potential of enhancing cavitation and complex drugs and nucleic acids to be distributed systemically. In cases where the ultrasonic strength is sine-shaped, the cavitation bubble switches between compression and rarefaction. The collapse of the cavitation bubble is rapid, occurring in the instance of the abrupt rise in ultrasonic strength, which violates the cell membrane by shock wave and by the minute jets. Gene transfer with the use ultrasound relies on the frequency of the transducer, acoustic pressure, pulse length, and time of exposure. The concentration and formulation of ultrasonic contrast agent are also a concern. Sonoporation has been done using diagnostic and specialized ultrasound equipment. The latter consists of a generator, amplifier, and air-backed, focused, or not piezoelectric transducer. This device enhances transfection by performing minor adjustments to physical parameters. Sonoporation was experimented with various cell types and tissues and has been demonstrated to be a less risky procedure of transferring plasmid DNA compared to electroporation. Medication and gene delivery to diseased tissues have now been done using sonoporation instead of tissue engineering. Sonoporation is better *in vivo* in the tissues that are close to the blood vessel. Sonoporation can work with tissue engineering in case the cavitation homogeneity of pore formation and cell contrast are enhanced.

Optoporation

Laser-induced gene therapy or optoporation uses a laser beam to perforate cell membranes. They assessed and plated the gene in a 0.5 mm spot size, 1mJ ultraviolet nanosecond laser, to the required cell. One hundred and 33 cells per minute could be transformed, 10 times more efficient in transfection than the manual technique and three orders of magnitude more efficient than the chemical technique, with at least 1 in 102 cells being changed. The laser beam is directed on the target cell by a lens. Predictably, local heat effect produces a change of cell membrane permeability on beam contact. Its efficiency hinges on the difference between the osmotic pressure of the cell and the medium, but this is sufficient to introduce a gene into a cell via the media.[29] Pulsed laser penetration into a cell a few times does not kill the cell. Computer-controlled laser light can be delivered using optical fibers, which have previously inaccessible body parts, more accessible were showed in Figure 4.

MAGNETIC FIELD ENHANCED TRANSPORT

Gene delivery is enhanced by Magnetoporation (magnetofection). In magnetoporation, cells are exposed to a magnetic field to deliver a nucleic acid. Magnetofection reagent and exogenous nucleic acids create a complex of biomolecule magnetic reagent. The chemical gets into the cell through magnetic field. Magnetic fields enhance

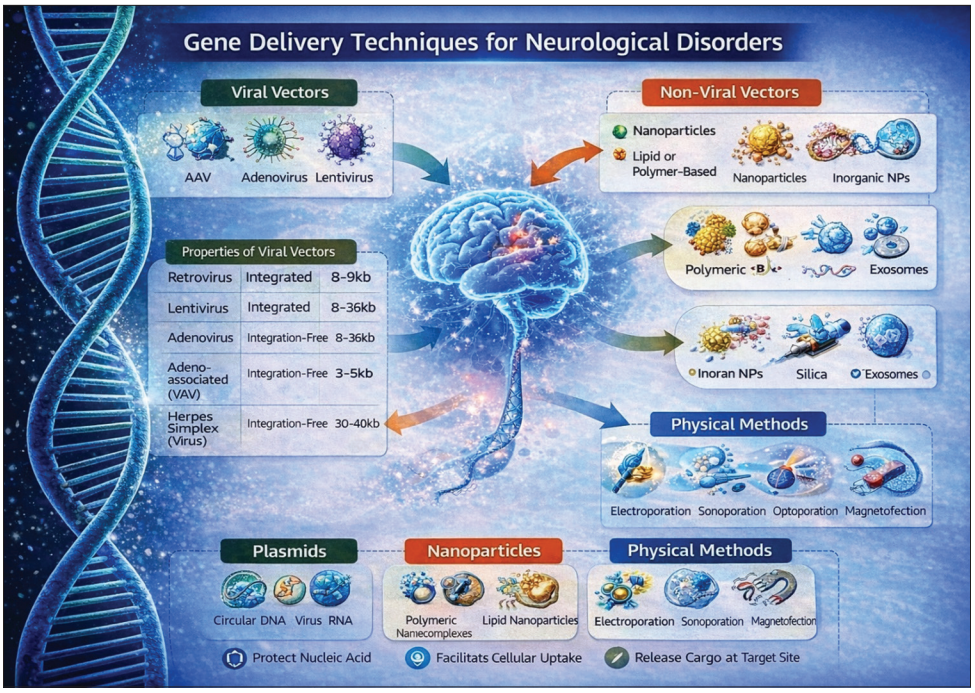

Figure 4: Gene delivery techniques for neurological disorders

pino and endocytosis of cell membrane. The concept is the combination of magnetic nanoparticles with DNA and transfection reagent or a viral vector. Magnetic nanoparticles are iron oxide polymer-coated biodegradable nanoparticles. Magnetofection can increase the efficiency of transfection 100-fold. The transfection of HUVEC cells, which are not easily transfected, was achieved with a gene reporter that expressed green fluorescent protein. This technology is capable of delivering genetic material to the target cell surface and inducing uptake since a magnetic field may induce extravasation of magnetic particles into the adjacent tissue or dragging them across the plasma membrane and into the cell. Field-guided, local, transfection in the gastrointestinal tract and blood arteries was proven to be efficient *in vivo*. It is a method used to deliver antisense oligonucleotides *in vitro* as well as *in vivo*. Magnetofection accelerates the entry of nucleic acids into the cell and nucleus, as well as enables reduced doses. Magnetofection is not better than gene delivery transfection.

CONCLUSION

Application of gene therapy in cancer, cardiovascular disease and neurological disease treatment has the potential to enhance the treatment outcomes and reduce the morbidity levels of the traditional dosage forms. New gene therapies models are set to play their role in the enhancement of life expectancy by reducing the symptoms of illness and direct treatment of diseases, rather than disease management. The application of multiple carriers as viral and non-viral vectors and physical procedures such as magnetoporation and electroporation may help transport genetic material through lessening the chances of degradation and leading to the delivery of the gene to the desired site of action. Gene therapy is promising to play a role in treating various illnesses as well as providing a potentially beneficial mitigation effect against adverse outcomes.

AUTHORS' CONTRIBUTIONS AND INVOLVEMENT

Dr. Balakrishna Talamanchi: Conceptualisation of the review topic, project supervision, manuscript design, critical revision, and final manuscript approval. Dr. Venkateswara Rao Javvaji and Dr Venkata Gopaiah Kurra: provides scientific direction, technological validation of gene therapy concepts, content review, and intellectual input to improve paper quality. Dr. Indira Avisa and Hasini Uddagiri: is responsible for collecting literature, preparing initial draft sections, and compiling gene delivery techniques. Lavanya Nagarapu: Helped write sections on viral and non-viral vectors and assisted with document layout. Shaik Fareedhunnisa: Conducts a literature review and prepares content on cancer gene therapy techniques. Shaik Abidunnisa: Contributed to sections on neurological disorders and gene therapy, as well as organising scientific references. Shabana Begam: Assisting with figure and diagram preparation, as well as reviewing therapeutic application parts. Devi Chandana Katari: contributed to cardiovascular gene therapy content and proofread the text. Naga Divya Yarlagadda: A compilation of recent advancements in nanoparticle-based gene delivery and reference formatting. Maha Lakshmi Veeranagula: provided

editing, proofreading, reference checking, and support with final manuscript preparation.

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